Storage Effects on galactosidase

7/14/49.

32 hour cells from 42 lac 9:35 to 2:30

Assay is azide.

A. 1½ml cells ¾ml buffer B. water. Incubate

C. " " D. " Refrigerate

	Initial: Di	Initial: Df.	Final: Di	Final: Df. 10 min.	
A	059	472	059	730	A.
B	061	242	061	109	
C	056	930	056	590	
D	060	241	060	160	

a). Note irregular excess of buffer treated cells over water treated. Buffer was M/10 Na. pH 7.5

Is activation related to Na^+?, λ? Assay in K buffer.

7/14/49.

PM. Hancock 10 hr. cells from Y2 Lac.

Dilute equal volume ē a) water b) NaP M/5 pH 7.5 c) KP do.
d) NaCl M/5 e) Sucrose M/5

.1 ml samples assayed.

$B^{30} - 10^{25}$

$D_{i\ 10\ min.}$	H_2O	084.	274
a		075	158.
b		042	>750 [5 mins.].
c		040	>750 [5 mins.].
d		066	410
e		071	375

[phosphate buffers, which also permit lysis, are most effective in augmenting activity.
pH effect? concentration? Measure pH's.

verify lysis by uv absorption of supernatant.

Suspensions A and B contain ca. 1.5 and 2.2 mg/ml respectively. [For ÷ .1 mg, use $\frac{1}{15}$ ml for A and $\frac{1}{22}$ ml for B.]

Assay .05 ml each.

	D_i	D_c	Δ_{cor}
A	018	18.4	155
B	030	430 (5 mins).	390 × 4.
Blank	001	014.	

−013 for substrate + 10% for dilution.

B) .11 mg had activity of $\frac{20}{5} \times 4 = 16$ u. ≐ 150 u/mg = full activity of the cells dried.

A) .075 mg had 1.5 u. ≐ 20 u/mg. ÷ full activity, not augmented.

Differences between treated and untreated cells persist on drying.

?? Can inactive, cell-free or dried preparations be activated?

Sediment A and B. Resuspend sediment in 5 ml H_2O (= 1) and keep supernatant (= 2). B2 is much more opalescent than A1.

Save samples; also mix A1, A2 etc. 1:1 = NaP M/5.

Incubate $3^{30} \rightarrow 5^{30}$

Test .1 ml samples A1, A2 and A1P, A2P.

	Pi	
A1	040	155 10
A2		M3 20
B1	068	530 5
B2		470 6
A1P	016	140 10
A2P		099 20
B1P	030	300 5
B2P		260 6

Y10 and Y70 grown on lactose. Incubate 1:1 with water, buffer M/10.

a) 420 — 700 Assay.

b) K-12 grown on lactose. Incubate 1:1 with water, buffer, etc.

K-12 / glucose [KG]. water, M/10 buffer.

1:1:1 lactose, 2%, water, M/100 buffer, M/10 buf.

	D:		
KG O	062	} negl. 10m.	
KG P	041		
KG-L-O	032		
L-P	030		
L-P	027		
KL-O	139	37'	6M
KL-P	111	520	6M
KL-P	078	>1150	5M
Y10-O	095	119	10M.
Y10-P	072	960	7M.
Y70-O	113	negl.	
Y70-P	076	152	9M.

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Lactase K-12 extract 2%. Activity ca 1200 u/ml.

.001 ml in M/50 NaP 7.5 M/2000 ONPG. Stop Rx ε Na_2CO_3

Alc	Conc	20m. Rdg.
—		119
Mannitol	M/10	132
Sorbitol	M/10	133
PrOH	M/100	119
"	M/10	134 ← Optimal concentration.
"	M/1	113
"	2M	029
"	5M	006

Recheck Mannitol and PrOH concentration. Also, L 341 which showed larger alcohol effects.

8/9/49 .002 ml, as above

1	—	
2	—	
3.	PrOH	M/10
4.	Et<OH OH	M/10
5.	Mannitol	M/10
6	~~BuOH~~	M/10